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L7: Entry 11 of 17 File: USPT May 19, 1998

DOCUMENT-IDENTIFIER: US 5753441 A
TITLE: 170-linked breast and ovarian cancer susceptibility gene

Drawing Description Paragraph Right (10):
FIG. 9B shows a mutation and cosegregation analysis in BRCA1 kindreds. Carrier individuals are represented as filled circles and squares in the pedigree diagrams. Frameshift mutation in Kindred 1910. The first three lanes are control, noncarrier samples. Lanes labeled 1-3 contain sequences from carrier individuals. Lane 4 contains DNA from a kindred member who does not carry the BRCA1 mutation. The diamond is used to prevent identification of the kindred. The frameshift resulting from the additional C is apparent in lanes labeled 1, 2, and 3.

Drawing Description Paragraph Right (11):
FIG. 9C shows a mutation and cosegregation analysis in BRCA1 kindreds. Carrier individuals are represented as filled circles and squares in the pedigree diagrams. Inferred regulatory mutation in Kindred 2035. ASO analysis of carriers and noncarriers of 2 different polymorphisms (PM1 and PM7) which were examined for heterozygosity in the germline and compared to the heterozygosity of lymphocyte mRNA. The top 2 rows of each panel contain PCR products amplified from genomic DNA and the bottom 2 rows contain PCR products amplified from CDNA. "A" and "G" are the two alleles detected by the ASO. The dark spots indicate that a particular allele is present in the sample. The first three lanes of PM7 represent the three genotypes in the general population.

Detailed Description Paragraph Right (24):
Once a sufficiently small region containing the BRCA1 locus was identified, physical isolation of the DNA in the region proceeded by identifying a set of overlapping YACs which covers the region. Useful YACs can be isolated from known libraries, such as the St. Louis and CEPH YAC libraries, which are widely distributed and contain approximately 50,000 YACs each. The YACs isolated were from these publicly accessible libraries and can be obtained from a number of sources including the Michigan Genome Center. Clearly, others who had access to these YACs, without the disclosure of the present invention, would not have known the value of the specific YACs we selected since they would not have known which YACs were within, and which YACs outside of, the smallest region containing the BRCA1 locus.

Detailed Description Paragraph Right (54):
Mismatches, according to the present invention, are hybridized nucleic acid duplexes in which the two strands are not 100% complementary. Lack of total homology may be due to deletions, insertions, inversions or substitutions. Mismatch detection can be used to detect point mutations in the gene or in its mRNA product. While these techniques are less sensitive than sequencing, they are simpler to perform on a large number of tumor samples. An example of a mismatch cleavage technique is the RNase protection method. In the practice of the present invention, the method involves the use of a labeled riboprobe which is complementary to the human wild-type BRCA1 gene coding sequence. The riboprobe and either mRNA or DNA isolated from the tumor tissue are annealed (hybridized) together and subsequently digested with the enzyme RNase A which is able to detect some mismatches in a duplex RNA structure. If a mismatch is detected by RNase A, it cleaves at the site of the mismatch. Thus, when the annealed RNA preparation is separated on an electrophoretic gel matrix, if a mismatch has been detected and cleaved by RNase A, an RNA product will be seen which is smaller than the full length duplex RNA for the riboprobe and the mRNA or DNA. The riboprobe need not be the full length of the BRCA1 mRNA or gene but can be a segment of either. If the riboprobe comprises only a segment of the BRCA1 mRNA or gene, it will be desirable to use a number of these probes to screen the whole mRNA sequence for mismatches.

Detailed Description Paragraph Right (78):

"Isolated" or "substantially pure". An "isolated" or "substantially pure" nucleic acid (e.g., an RNA, DNA or a mixed polymer) is one which is substantially separated from other cellular components which naturally accompany a native human sequence or protein, e.g., ribosomes, polymerases, many other human genome sequences and proteins. The term embraces a nucleic acid sequence or protein which has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates and chemically synthesized analogs or analogs biologically synthesized by heterologous systems.

Detailed Description Paragraph Right (193):
We further showed that the BRCA1 locus must lie distal to the marker Mfd191 (D17S776) (Goldgar et al., 1994). This marker is known to lie distal to THRA1 and RARA. The smallest published region for the BRCA1 locus is thus between D17S776 and D17S78. This region still contains approximately 1.5 million bases of DNA, making the isolation and testing of all genes in the region a very difficult task. We have therefore undertaken the tasks of constructing a physical map of the region, isolating a set of polymorphic STR markers located in the region, and analyzing these new markers in a set of informative families to refine the location of the BRCA1 gene to a manageable interval.

Detailed Description Paragraph Right (208):
Those clones that contain HTF islands or show hybridization to other species DNA besides human are likely to contain coding sequences. The human DNA from these clones was isolated as whole insert or as EcoRl fragments and labeled as described above. The labeled DNA was used to screen filters of various cDNA libraries under the same conditions as the zoo blots except that the CDNA filters undergo a more stringent wash of 0.1.times.SSC, 0.1% SDS at 65.degree. C. for 30 minutes twice.

Detailed Description Paragraph Right (218):
To screen for mutations in the affected pedigrees, two different approaches were followed. First, genomic DNA isolated from family members known to carry the susceptibility allele of BRCA1 was used as a template for amplification of candidate gene sequences by PCR. If the PCR primers flank or overlap an intron/exon boundary, the amplified fragment will be larger than predicted from the cDNA sequence or will not be present in the amplified mixture. By a combination of such amplification experiments and sequencing of P1, BAC or cosmid clones using the set of designed primers it is possible to establish the intron/exon structure and ultimately obtain the DNA sequences of genomic DNA from the pedigrees.

Detailed Description Paragraph Right (261):
Membranes are UV cross linked at 150 mJ using a GS Gene Linker (Bio-Rad). BRCA1 probe corresponding to nucleotide positions 3631-3930 of SEQ ID NO:1 is subcloned into pTZ18U. The phagemids are transformed into E. coli MV1190 infected with M13KO7 helper phage (Bio-Rad, Richmond, Calif.). Single stranded DNA is isolated according to standard procedures (see Sambrook et al., 1989).

CLAIMS:

7. The method of claim 1 wherein a germline nucleic acid sequence is compared by hybridizing a BRCA1 gene probe which specifically hybridizes to a BRCA1 allele to genomic DNA isolated from said sample and detecting the presence of a hybridization product wherein a presence of said product indicates the presence of said allele in the subject.

11. The method of claim 1 wherein a germline nucleic acid sequence is compared by obtaining a first BRCA1 gene fragment from (a) BRCA1 gene genomic DNA isolated from said sample, (b) BRCA1 RNA isolated from said sample or (c) BRCA1 CDNA made from mRNA isolated from said sample, obtaining a second BRCA1 gene fragment from (a) wild-type BRCA1 genomic DNA, (b) wild-type BRCA1 RNA or (c) wild-type cDNA made from wild-type mRNA, said second BRCA1 gene fragment corresponding to said first BRCA1 gene fragment, forming single-stranded DNA from said first BRCA1 gene fragment and from said second BRCA1 gene fragment, forming a heteroduplex consisting of single-stranded DNA from said BRCA1 gene fragment and single-stranded DNA from said second BRCA1 gene fragment, analyzing the heteroduplex to determine if said single-stranded DNA from said first BRCA1 gene fragment has a mismatch relative to said single-stranded DNA from said

second BRCA1 gene fragment and sequencing said first single-stranded DNA from said first BRCA1 gene fragment having a mismatch.

22. The method of claim 20 wherein a germline alteration is detected by hybridizing a BRCA1 gene probe which specifically hybridizes to one of said alterations to genomic DNA isolated from said sample and detecting of the presence of a hybridization product, wherein the presence of said product indicates the presence of said alteration in the sample.

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L5: Entry 10 of 11 File: USPT Nov 4, 1997

DOCUMENT-IDENTIFIER: US 5684242 A
TITLE: Nuclear restorer genes for hybrid seed production

Brief Summary Paragraph Right (2):
In plants, the best first filial (F.sub.1) hybrids have a substantial yield advantage over the best open-pollinated varieties or inbred lines. This yield advantage of a hybrid over its parents is termed heterosis. The degree of heterosis observed varies among species; however, as a general rule, it is high among cross-pollinated species, such as maize and sunflower, and typically lower among self-pollinated species, such as soybean and wheat. Indeed, providing the generation of producing the hybrid seed is sufficiently inexpensive, the manufacture and sale of hybrid seed forms the basis of a significant agricultural industry. For example, hybrid varieties of corn (a/k/a maize) largely supplanted open-pollinated varieties at least from the 1940's because of the ease of generating hybrid maize seed coupled to the startling improvement in yield and other agronomic traits when hybrid varieties were used.

Brief Summary Paragraph Right (4):
In maize, an intermediate course historically has been taken: It is possible to grow two maize parents in isolation from other maize pollen sources, detassel (emasculate) the "female" parent plants and let the "male" parent produce pollen and fertilize via standard cross-pollination. The designation of male and female may be considered arbitrary in this context because wild-type maize is monecious, having both male and female flowers on each plant. However, there often are commercially significant reasons for selecting one parent rather than the other as female or male. For example, a preferred female has ample seed production, whereas a preferred male has ample pollen production. Seed harvested from the female parent is therefore derived from the cross of the female by the male and thereby produces hybrid seed.

Brief Summary Paragraph Right (6):
A maize variety can be converted into a CMS line by crossing it (as male) to a known cytoplasmic male sterile line and then backcrossing it (as female) to the initial inbred line. Of course, the CMS-converted line is male sterile, so it must be maintained by crossing by the original inbred line (called a maintainer). To make hybrid seed, one merely grows the CMS-converted inbred line and a second inbred line in isolation. Because the CMS-converted line is male-sterile, it is not necessary to detassel it. If the second inbred line is also CMS-converted, the hybrid progeny will also be male sterile, resulting in infertile plants that would have limited economic value. To overcome this difficulty, nuclear restorer genes are used. Most male sterile cytoplasms are restored to fertility in a succeeding generation when combined with certain nuclear genes, called restorers. If the second inbred line carries one of these restorers, then the F.sub.1 will be male-fertile, and, potentially, provide economic value.

Brief Summary Paragraph Right (7):
In maize (Zea mays L.), there are three major groups of male-sterile cytoplasms: S (USDA), C (Charrua), and T (Texas), in addition to the N or normal male-fertile cytoplasm. These cytoplasms can be classified by the different nuclear genes that suppress their associated male-sterile phenotype, thereby allowing normal pollen development (see Laughnan et al., supra), by mitochondrial DNA restriction endonuclease profiles (see Pring et al., Genetics, 89, 121-136 (1978)), and by characteristic polypeptide patterns resulting from .sup.35 S-methionine incorporation by isolated mitochondria (see Forde et al., Proc. Nat'l. Acad. Sci. USA, 75, 3841-3845 (1978)).

Brief Summary Paragraph Right (8):
The normal N cytoplasm yields fertile plants in either the presence or absence of all

known nuclear backgrounds, whereas the male-sterile C, S, and T cyto-plasms only produce fertile plants in nuclear backgrounds carrying the appropriate restorer genes. These nuclear-encoded fertility-restorer genes compensate for cytoplasmic dysfunctions that are phenotypically expressed during microsporogenesis and/or microgametogenesis. Plants carrying S and C cytoplasm are restored to fertility by single dominant alleles of the rf3 and rf4 loci, respectively. The rf4 locus maps to chromosome 8, approximately 2 cM from the RFLP ("restriction fragment length polymorphism") marker NP1114A (Sisco, Crop Sci., 31, 1263-1266 (1991)). Preliminary evidence suggests that the rf3 locus is flanked by whp and bn117.14 on chromosome 2 L (T. Kamps et al., Maize Genet. Coop. Newsl., 66, 45 (1992)). In contrast to S and C cytoplasm, plants with T cytoplasm are restored to fertility by the dominant alleles of two loci, rf1 and rf2 (Laughnan et al., supra), which are located on separate chromosomes. The rf1 locus is flanked by umc97 and umc92 on chromosome 3 and the rf2 locus is flanked by the umc153 and sus1 on chromosome 9 (Wise et al., Theor. Appl. Genet., 88, 785-795 (1994)).

Brief Summary Paragraph Right (10):
Maize is not alone in having a CMS phenomenon. Other species also have approaches to engineering male sterility, which include a variety of cytoplasmic male sterility (CMS) and fertility restoration systems that have been well characterized at the genetic and molecular levels. Some examples of these CMS systems include petunia (Nivison et al., Plant Cell, 1, 1121-1130 (1989)), common bean (Janska et al., Genetics, 135, 869-879 (1993)), Brassica napus (Singh et al., Plant Cell, 3, 1349-1362 (1991)), sunflower (Laver et al., The Plant Journal, 1, 185-193 (1991)), sorghum (Bailey-Serres et al., Theor. Appl. Genet., 73,252-260 (1986)), oats (Mann et al., Theor. Appl. Genet., 78, 293-297 (1989)), S-cytoplasm maize (Schardl et al., Cell, 43, 361-368 (1985)), and T-cytoplasm maize (Levings, Plant Cell, 5, 1285-1290 (1993)). Cytoplasmic male sterility in petunia, beans, Brassica, and S-cytoplasm maize can be restored to fertility by single dominant nuclear genes. In contrast, T-cytoplasm maize is restored by the combination of dominant alleles of two unlinked, nuclear restorer genes, rf1 and rf2 (Laughnan et al., supra).

Brief Summary Paragraph Right (11):
Most of the research on CMS systems has focused on the characterization of novel open reading frames in their respective mitochondrial genomes, i.e., the cytoplasmic component of CMS. Such research has revealed that, although each open reading frame is unique, all known such open reading frames appear to have large hydrophobic domains (Dewey et al., Proc. Nat'l. Acad. Sci. USA, 84, 5374-5378 (1987)). In T-cytoplasm maize, the unique mitochondrial gene, T-urf13, is associated with the CMS (Wise et al., Proc. Nat'l. Acad. Sci. USA, 84, 2858-286 (1987a)) and toxin sensitivity traits (Huang et al., EMBOJ, 9, 339-247 (1990)). T-urf13 encodes a 13 kDa mitochondrial polypeptide (URF13) (Wise et al., Plant Mol. Biol., 9, 121-126 (1987b)), located in the mitochondrial membrane (Dewey et al., supra (1987)). It is also known that this polypeptide is not synthesized by deletion mutants (Dixon et al., Theor. Appl. Genet., 63, 75-80 (1982)), and is truncated in a T4 frameshift mutant (Wise et al., supra (1987b)). The URF13 protein binds to fungal pathotoxins (Braun et al., Plant Cell, 2, 153-161 (1990)) and appears to span the mitochondrial membrane in oligomeric form (Korth et al., Proc. Nat'l. Acad. Sci. USA, 88, 10865-10869 (1991).

Brief Summary Paragraph Right (13):
T cytoplasm was used predominantly in the late 1960's because of its reliability to cause male sterility in the female plants. The other CMS systems of maize, the C and S cytoplasms, tended to "break down" in the field, allowing some self-fertilization by the female plants or failing to restore completely. Thus, approximately 85% of the U.S. hybrid maize seed had a genetic background that included T cytoplasm until the 1970 epidemic of southern corn leaf blight (Pring et al., Ann. Rev. Phytopathol., 27, 483-502 (1989)). Never before or since has a major crop in the United States had so nearly uniform a genetic background underlying the various hybrids on the market at that time. Subsequent to the 1970 epidemic, it was determined that maize that carries T cytoplasm is highly sensitive to the host-selective toxin (T toxin) produced by race T of the fungus Cochliobolus heterostrophus Drechsler (asexual stage Bipolaris mayadis Nisikado and Miyake), which is the causal organism of southern corn leaf blight. See Comstock et al., Phytopathology, 63, 1357-1361 (1973). T cytoplasm-carrying maize was also found to be highly sensitive to the host-selective toxin (Pm toxin) produced by another fungus Phyllosticta maydis, Army and Nelson, which causes yellow leaf blight (Yoder, Phytopathology, 63, 1361-1366 (1973)). Accordingly, the major seed producers

have resorted to a system of selection that involves using different CMS systems (including T). Hybrid seed produced using these different systems is mixed together prior to being sold to farmers. In this way, any given farmer's fields are sown with seeds that include varying combinations of, for example, T, C, S, and N cytoplasms produced either via CMS (in the case of T, C, and S) or detasseling (in the case of N).

Brief Summary Paragraph Right (14):
A focus of research since the 1970's has been to develop alternative genetic approaches to emasculating plants for the purpose of hybrid seed production. This effort is due in part to the interest of not having an homogeneous genetic background in crops. One such system, invented by Marc Albertsen for Pioneer Hi-Bred International, Inc., involves using a genetic male sterility system located in the nuclear genome. There are many such mutations available in a given plant species, including maize (Albertsen et al., Can. J. Genet. Cytol, 23, 195-208 (1981)). The Pioneer system is based on a molecular clone of the nuclear maize gene that confers male sterility, and is predicated, at least in part, on earlier analogous work on Arabidopsis (see Aarts et al., Nature, 363, 715-717 (1993)). The difficulty of this approach centers on the maintenance of the male sterile ("ms") line. To overcome this difficulty, Albertsen has proposed the creation of an inbred line that is homozygous for a mutant allele of the ms gene. This line is proposed also to carry a genetically engineered construct that has an inducible promotor that will allow expression of a wild-type coding region of the ms gene. To maintain the inbred line, it will be grown by itself in isolation, sprayed with the inducer (which turns on the wild-type ms gene), and allowed to self- and sib-pollinate. To make hybrid seed the inbred line will be grown in isolation with a second inbred line in the absence of inducer, thus the first line will be male sterile and hybrid seed will be harvested from it. As used in the farmers' fields, the F.sub.1 should be male fertile because the second inbred line of the cross will carry a wild-type allele of the ms gene, making the F.sub.1 heterozygous, and thus fertile.

Brief Summary Paragraph Right (15):
Another system designed to augment the prior CMS systems was developed by Leemans et al. for Plant Genetic Systems ("PGS"), and was disclosed in Nature, 347, 737-741 (1990). The PGS system is based on an RNase gene that is driven by a tapetum-specific promotor. The RNase is therefore active only in the anthers where it kills the tapetum, which are structures that normally nourish the pollen. The result is male-sterile plants. This system results in dominant male-sterility. Hence by introducing (via backcrossing or transformation) this system into an inbred line, one generates a male sterile inbred line. However, the mutation is heterozygous and therefore when the male-sterile line is crossed by a normal line, the progeny segregate for ms and N (i.e., normal). To overcome this difficulty, the Plant Genetic Systems method uses a genetic construct whereby a herbicide resistance gene is linked tightly to the ms gene. In practice, the ms line is crossed by the normal progenitor line. The resulting segregating progeny are grown in isolation with a second inbred line. The rows that carry the first inbred line are sprayed with an herbicide. The male fertile progeny die, leaving only the ms inbred plants from which hybrid seed can be harvested.

Brief Summary Paragraph Right (17):
Accordingly, it is an object of the present invention to provide new materials and methods that will allow one to produce hybrid seeds from a female line having novel cytoplasmic male sterility factors. It is a further object of the present invention to provide new plants having novel cytoplasmic male sterility factors and/or plants producing seeds having novel cytoplasmic male sterility factors. It is yet a further object of the present invention to provide the isolated genes and gene products responsible for the cytoplasmic male sterility factors in plants.

Brief Summary Paragraph Right (19):
It has been discovered that nuclear restorer genes may be isolated and used for the production of new varieties of CMS systems. Accordingly, an enriched or substantially isolated nucleic acid comprising a nucleotide sequence that encodes a nuclear male fertility restorer gene, in particular, has been isolated. Vectors, organisms, seeds, plant cells, and plants comprising such a nucleic acid have been discovered as well.

Detailed Description Paragraph Right (2):
In particular, the present invention provides an enriched or isolated nucleic acid comprising a nucleotide sequence that encodes a nuclear restorer gene product of a plant, or a portion thereof, the action of which is to restore male fertility to a plant having a cytoplasmic male-sterility (CMS) trait. As used herein, the CMS trait is considered to be the direct result of certain CMS factors, which may actually be a single or multiple genes acting to produce the CMS trait. A nuclear restorer gene product may be a polypeptide or an RNA molecule. The nuclear restorer gene product, as just stated, is involved in restoring male fertility by action on mitochondrial or chloroplast functions, although the present invention is derived from the nuclear genome.

Detailed Description Paragraph Right (3):
Genes in maize that effect restoration of cytoplasmic male sterility have been named "rf". See Levings et al., Cell, 56, 171-179 (1989). Similar effects have been noted in a range of other plant species, as noted in the Background, and are believed to be applicable to a broad range of plants including such important agricultural crops as soybean, alfalfa, wheat, sorghum, beet, various vegetables including cucumber, tomato, peppers, and the like, various trees including apple, pear, peach, cherry, redwood, pine, oak, and the like, and various ornamentals. Indeed, in view of the fundamental nature of the activity of the present invention, the present invention is viewed as applicable to any sexual reproducing plant.

Detailed Description Paragraph Right (4):
The term "nucleic acid" refers to a polymer of DNA or RNA, i.e., a polynucleotide, which can be single- or double-stranded, and can optionally contain synthetic, nonnatural, or altered nucleotides. Any combination of such nucleotides can be incorporated into DNA or RNA polymers. The nucleic acid is "enriched" in that the concentration of the material is at least about 2, 5, 10, 100, or 1000 times its natural concentration, for example, advantageously 0.01% by weight, preferably at least about 0.1% by weight. Enriched preparations of about 0.5%, 1%, 5%, 10%, and 20% by weight are also contemplated. A polynucleotide is "isolated" in that the material has been removed from its original environment, e.g., the genome of a plant, presuming that it is naturally occurring. Thus, describing the polynucleotide of the present invention as "substantially isolated" reflects the increase in concentration of the polynucleotide of interest with respect to other polynucleotides, as when, for example, the polynucleotide of interest is taken from a plant (which, in the case of maize, has a complement of three million kb) and is placed or cloned into a bacteriophage (which, in the case of lambda (".lambda.") has a complement of 50 kb), resulting in a 60,000-fold increase in concentration of the inventive polynucleotide sequence with respect to the total amount of DNA in the bacteriophage in which it is placed. It is also advantageous that the nucleic acids be in purified form, or substantially purified form, wherein "purified" does not mean absolute purity but rather relative purity, wherein, for example, the nucleic acids of the present invention are isolated in a laboratory vessel in a mixture of other nucleic acids, such as portions of a vector or other molecules associated with genetic engineering.

Detailed Description Paragraph Right (5):
Preferably, the enriched or substantially isolated nucleic acid hybridizes under at least moderately stringent hybridization conditions to a second nucleic acid that includes nucleotide sequences specific to an rf gene, or substantial portions thereof; more preferred, the nucleic acid hybridizes under the aforementioned conditions to a second nucleic acid that includes nucleotide sequences specific to the rf1 or rf2 gene, or substantial portions thereof; yet more preferred the selected nucleotide sequence are specific to the rf2 gene.

Detailed Description Paragraph Right (8):
Four loci of the nuclear restorer (rf) genes of maize have been described in the technical literature (for example, see Levings et al., supra), and have been further elucidated by work disclosed herein. The rf loci affect the capability to restore male fertility when certain cytoplasmic genes are operating or not operating. The names of the known loci are rf1, rf2, rf3, and rf4. Plants having dominant rf genes (recited as Rf) are usually identified because the anthers are fully formed and functional in the presence of cytoplasmic male sterility factors.

Detailed Description Paragraph Right (9):
As noted above, with respect to the T cytoplasm, both rf1 and rf2 must have dominant alleles present to overcome the sterility factors provided by the T CMS system. Accordingly, a seedling must be at least heterozygous dominant for both loci before it counteracts a CMS T phenotype. Similarly, rf3 and rf4 have been identified as having analogous restoring functions in the, respectively, C and S CMS systems. The existence of the various rf genes involved in the restoration function suggests that the rf genes or a subset thereof may represent a gene family, wherein the members are related to an ancestral locus that duplicated and diverged in sequence at least once over evolutionary time. The importance of this observation and hypothesis is that the identification of one nucleic acid sequence specific to one of the rf genes, which is disclosed herein, may provide probes therefrom for the identification and isolation of other rf genes.

Detailed Description Paragraph Right (11):
The nucleic acid of the present invention can be isolated from any suitable plant having the appropriate genotype. As noted in the Background section above, all sexual plants have anthers, which in maize becomes incorporated in the tassel structure. Certain deficiencies of mitochondria have been shown to interfere with anther structure and, concomitantly, with pollen production. Because mitochondria are the energy providers to a cell, such deficiencies can be severe. Thus, such effects on male fertility can only be reversed if the cells can provide a means to reverse the mitochondrial deficiencies. As exemplified below, it is now known that at least one nuclear restorer gene (rf2) that counteracts cytoplasmic male sterility is isolable from a cDNA library derived from the mRNA present in tassel tissue. Construction of such a cDNA library and the appropriate harvesting of the tassel tissue was accomplished using conventional knowledge and techniques, using bacteriophage lambda as host for the cDNA library.

Detailed Description Paragraph Right (12):
The isolated rf2 cDNA sequence has a high degree of homology to aldehyde dehydrogenase from various sources, including cows and rats. Aldehyde dehydrogenase is a rather mundane "housekeeping" protein that acts in the mitochondria or cytosol. It is also an enzyme known to provide a detoxifying function, as utilized in the liver of a mammal. Such mechanisms can be foreseen to work and function in the same manner in essentially all plants, albeit the detoxifying function would necessarily take place in the absence of a liver. This is particularly supported by the fact, exemplified below, that the maize rf2 gene is highly conserved with respect to the same enzyme in cows, which, of course, are very distant from plants on the evolutionary scale.

Detailed Description Paragraph Right (13):
Accordingly, preferred nucleic acids of the present invention are isolated from any suitable sexual-reproducing plant. Such plants are either monocotyledonous or dicotyledonous, including maize, wheat, barley, rice, oats, rye, soybean, rapeseed, canola, cotton, safflower, peanut, palm, sorghum, sunflower, tomato, cucumber, and ornamental flowers. The more preferred plant that the rf genes are derived from is maize.

Detailed Description Paragraph Right (14):
The present invention also provides a nucleic acid of which the nucleotide sequence thereof hybridizes to the cDNA having the sequence of SEQ ID NO:1 or SEQ ID NO:3, or a sequence complementary thereto, under at least moderately stringent conditions. SEQ ID NO:1 and SEQ ID NO:3 encompass the same number of nucleotides, wherein SEQ ID NO:1 includes the entire sequence of SEQ ID NO:3, which includes two fewer nucleotide positions occupied by N, as provided in the SEQUENCE LISTING below. A preferred nucleic acid according to the present invention hybridizes to SEQ ID NO:1 only under stringent hybridization conditions. A more preferred enriched or isolated nucleic acid of the present invention comprises a nucleotide sequence selected from the group consisting of SEQ ID NO:1, a sequence complementary thereto, and a substantially equivalent sequence. SEQ ID NO:1 is a partial sequence (402 bases sequenced out of a total of about 1200 bases) of the cDNA derived from the maize rf2 gene, the identification and isolation of which are disclosed in Examples 1 and 2. SEQ ID NO:1 may not be a full-length cDNA (i.e., including the complete complement of the rf2 mRNA) because, as compared to the evidently homologous aldehyde dehydrogenase gene sequences disclosed at Example 4, approximately 500 bp of the rf2 message is missing.

Nevertheless, one of ordinary skill in the art certainly recognizes that SEQ ID NO:1 or SEQ ID NO:3 provides sufficient rf2 sequence information to provide probes for the identification and cloning of any rf2 genes or to any gene that is substantially homologous to rf2 in sequence such that the rf2 probe hybridizes under moderately to extremely stringent hybridization conditions, as discussed hereinabove.

Detailed Description Paragraph Right (16):
The enriched or isolated nucleic acid which comprises the nucleotide sequence of SEQ ID NO:1 is most preferred. The present inventive nucleic acid may be identified for enrichment or isolation by hybridization to any subfragment of SEQ ID NO:1 of at least 20 nucleotides under stringent hybridization conditions as described in Sambrook et al., Molecular Cloning: A Laboratory Mammal (2d ed., 1989). Accordingly, this invention encompasses the entire sequence of the rf2 gene and fragments thereof, which have been generated by any suitable technique, such as by restriction enzyme digestion of chromosomal or plasmid DNA, or by synthesis, and which may be either DNA or RNA.

Detailed Description Paragraph Right (17):
In addition to the methods recited in Example 1 for the identification and isolation of nuclear restorer genes and related nucleic acids of the present invention, other methods may be used alternatively, such as, inter alia, chromosome walking and heterologous probe selection.

Detailed Description Paragraph Right (18):
Chromosome walking is a particularly useful technology that can facilitate the molecular isolation of any mapped gene (Bender et al., J. Mol. Biol., 168, 17-338 (1983)) and has been found to be particularly useful with plants that have a relatively small genome size, such as that of Arabidopsis. Meyerowitz, in Methods in Arabidopsis Research (Koncz et al., eds., World Scientific, Singapore, 1992), pages 100-118). In addition, five YAC libraries, representing 28 genome equivalents, exist for this species. Gibson and Somerville, in Methods in Arabidopsis Research (supra). Several Arabidopsis genes have been cloned via this strategy (Yanofsky et al., Nature, 346, 35-39 (1990); Giraudat et al., Plant Cell, 4, 1251-1261 (1992); and Arondel et al., Science, 258, 1353-1354 (1992)). This technique is useful, of course; in species of larger genome size as well, such as maize.

Detailed Description Paragraph Right (19):
A chromosome walk is initiated by identifying from a library of large DNA fragments the specific fragment(s) that contain sequences homologous to a restriction fragment length polymorphism (RFLP) marker or some other marker that is closely linked to the target gene. Typically, the library of DNA fragments is maintained as yeast artificial chromosomes, i.e., YACs (Burke et al., Science, 236, 806-811 (1987)), although cosmids, P1 phage or .lambda. phage have been used. Single copy sequences from the termini of YACs that contain sequences homologous to a starting RFLP are then used as hybridization probes to isolate overlapping DNA fragments. This process is repeated until the entire chromosomal region, from the starting RFLP marker to beyond the target gene, has been cloned as a contiguous segment (a "contig"). Typically, the contig is oriented by mapping DNA sequences from the growing contig to the genetic/RFLP map. Similarly, the endpoint of the walk is established by demonstrating that the contig contains DNA sequences from both sides of the target gene. For both of these operations, DNA sequences from the contig must be genetically mapped. The efficiency of this mapping can be greatly increased by selecting a population of plants that have a high probability of carrying recombination breakpoints in the region defined by the contig. Such a mapping population is established by selecting plants that carry a recombination breakpoint between two visible genetic markers that flank the interval to be walked. The precision of the mapping increases proportionally with the number of genetic recombinants. The greater the precision of this mapping, the smaller the uncertainty associated with the positioning of the target gene on the contig. Once the target gene has been localized in the contig to as small an interval as the mapping population permits, the target gene is identified from the interval via its ability to complement genetically the mutant phenotype. The ability of a sequence to complement the mutant phenotype is assayed by transforming plants homozygous for a mutant allele of the target gene. Alternatively, comparisons between wildtype and mutant sequences can also identify the target gene from the interval.

Detailed Description Paragraph Right (20):

Other technologies for gene isolation in Arabidopsis and other plants include genomic subtraction, and transposon and T-DNA tagging. Genomic subtraction requires the availability of strains having deletions of the target gene (Strauss and Ausubel, - Proc. Natl. Acad. Sci. USA, 87, 1889-1893 (1990); Sun et al., Plant Cell, 4, 119-128 (1992)); however, such deletions are not available for rf2, for example. A transposon tagging system in Arabidopsis has recently become available. The success in tagging and cloning a petunia gene using a heterologous maize transposon (Chuck et al., Plant Cell, 5, 371-378 (1993)) provided the direction to extend this technique to Arabidopsis, and further supports the view that this technique is amenable to tagging virtually any plant with heterologous (Dean et al., Plant J., 2, 69-81 (1992); Grevelding et al., Proc. Natl. Acad. Sci. USA, 89, 6085-6089 (1992); Swinburne et al., Plant Cell, 4, 583-595 (1992); and Fedoroff and Smith, Plant J., 3, 273-289 (1993)) and/or endogenous (Tsay et al., Science, 260, 342-344 (1993)) transposons. T-DNA tagging, another method to locate a gene, has been realized (Feldmann, Plant J., 1, 71-82 (1991)) and is in wide use (e.g., Feldmann et al., Science, 243, 1351-1354 (1989); Herman et al., Plant Cell, 11, 1051-1055 (1989); Konz et al., EMBO J., 9, 1337-1346 (1989); Kieber et al., Cell, 72, 427-441 (1993)). Additionally, having isolated at least one nuclear restorer gene, the nucleic acid thereof can be used whole or in parts (by sub-cloning fragments thereof) as a probe in heterologous systems. Preferably, such a technique requires that the stringency of the selective hybridization procedure by lowered, and then slowly raised, as is well known in the art.

Detailed Description Paragraph Right (21):
Although T-DNA tagging, chromosome walking or heterologous probe selection can identify a DNA fragment that putatively contains the gene of interest, in each instance these DNA fragments must be confirmed by genetic complementation or some other means, which is fully disclosed in Examples 1 and 2. Although the methods of identification of a particular gene sequence has been described herein largely with reference to maize and Arabidopsis only, it is abundantly clear to one of ordinary skill that such methods may be adapted for gene identification in other species, particularly in the context of the present invention. Accordingly, the identification of the rf genes, and cloning and using thereof, is enabled hereby for any of the aforementioned sexual-reproducing plants, as well as other plants that have mitochondrial deficiency-derived phenotypes in need of amelioration or correction.

Detailed Description Paragraph Right (22):
The nucleic acids of the present invention may be cloned in any suitable vector and the vector as constructed with nucleic acid insert of the present invention is used to transform or transfect any suitable host. E. coli, in particular E. coli TB-1, TG-2, DH5.alpha., XL-BLue MRF' (Stratogene), SA2821 or Y1090, is a preferred host. A more preferred host is XL-Blue MRF' or TG-2. Suitable vectors include those designed for propagation and expansion or for expression or both. Constructs of vectors can be prepared, either circular or linear, to contain the entire rf gene nucleotide sequence or a portion thereof ligated to a replication system functional in a microorganism host, whether prokaryotic or eukaryotic. Suitable hosts include E. coli, B. subtilis, P. aerugenosa, S. cerevisiae, and N. crassa. Replication systems may be derived from ColE1, 2 m.mu. plasmid, lambda, SV40, bovine papilloma virus, or the like. In addition to the replication system and the inserted DNA, the construct usually will include one or more markers, which allow for selection of transformed or transfected hosts. Markers may include biocide resistance, e.g., resistance to antibiotics, heavy metals, etc., complementation in an auxotrophic host to provide prototrophy, and the like. A preferred cloning vector is selected from the group consisting of pUC18, pET11d, EMBL4, NM1149, pLZO3 and Lambda ZapII (Stratagene). Of course, it is well-appreciated in the art that nucleic acids may be cloned in suitable bacteriophage vectors, such as, for example .lambda.GT10.

Detailed Description Paragraph Right (25):
Care should be taken to choose a vector that does not result in cytotoxic expression of the amino acid sequence encoded in the insert of the vector. For expression in microorganisms, the expression vector may differ from the cloning vector in having transcriptional and translational initiation and termination regulatory signal sequences and may or may not include a replication system that is functional in the expression host. The coding sequence is inserted between the initiation and termination regulatory signals so as to be under their regulatory control. Expression

vectors may also include the use of regulatable promoters, e.g., temperature-sensitive or inducible by chemicals, or genes that allow for integration and amplification of the vector and insert DNA, such as tk, dhfr, metallothionein, and the like. Such controls, if incorporated into a plant, could allow for the efficient and economic production of hybrid seeds, for example, by promoting expression of the restorer gene function upon the advent of a triggering level of some inducing substance.

Detailed Description Paragraph Right (26):
The vector can be used to express a dsDNA sequence, either isolated and cloned or synthesized, to obtain a precursor protein, which is subject to further manipulation by cleavage, a complete mature protein, or a fragment thereof by introducing the expression vector into an appropriate host, where the regulatory signals are functional in the host. The expression host is grown in an appropriate nutrient medium, whereby the desired polypeptide is produced and isolated from cells or from the medium, when the polypeptide is secreted. Where a host is employed in which the vector's transcriptional and translational regulatory signals are functional, then the rf gene DNA sequence may be manipulated to provide for the expression of the desired polypeptide in proper juxtaposition to the regulatory signals. The polypeptide products can be obtained in substantially pure form, particularly free of cellular debris, which may include such contaminants as, for example, proteins, polysaccharides, lipids, nucleic acids, viruses, bacteria, fungi, and combinations thereof, using methods well known in the art.

Detailed Description Paragraph Right (28):
The method of detection involves duplex formation by annealing or hybridization of the oligonucleotide probe, either labeled or unlabeled, depending upon the nature of the detection system, with the DNA or RNA of an organism believed to produce the particular nuclear restorer gene. Usually this method of detection involves cell lysis, extraction of nucleic acids with organic solvents, precipitation of nucleic acids in an appropriately buffered medium, and isolation of the DNA or RNA. Alternatively, one can amplify specific sequences via polymerase chain reaction (PCR). The DNA may be fragmented by mechanical shearing or restriction endonuclease digestion. The nucleic acid may then be bound to a support or may be used in solution depending upon the nature of the protocol. The Southern technique (Southern, J. Mol. Biol., 98, 503 (1975)) may be employed with denatured DNA by binding the single-stranded fragments, for example, to a nitrocellulose or nylon filter. RNA also may be blotted onto a filter (Thomas, Proc. Natl. Acad. Sci. USA, 77, 5201 (1980)). Preferably, the fragments are subjected to electrophoresis prior to binding to a support so as to enable the selection of various sized fractions. Alternatively, the assay may be accomplished on plant cells fixed to a substrate and permeabilized using methods known in the art, whereupon the hybridization procedure can be conducted to determine if a homologous gene to a particular nuclear restorer gene exists in the plant of interest, and/or if that plant is expressing RNA that is homologous to the nuclear restorer gene.

Detailed Description Paragraph Right (30):
In accordance with another aspect of the present invention, the nucleic acids disclosed herein are used in a method for the production of hybrid seed, comprising the steps of: (a) constructing a nucleic acid comprising a nucleotide sequence that encodes a male fertility restorer gene product; (b) inserting the nucleic acid into a plant cell; and (c) establishing a plant from the plant cell having and expressing the nucleic acid. The first plant, which is one of a line or variety of such plants, preferably can produce seeds, however, such seeds may or may not be fertile. Accordingly, the inventive method involves plant tissue culture techniques known in the art. Insertion of nucleic acids into a plant cell is accomplished by any suitable means, including cell bombardment, i.e., attaching the DNA to metallic pellets and blasting them through the plant's cell wall (Fromm et al., Bio/Technology, 8, 833-839 (1990); Gordon-Kamm et al., Plant Cell, 2, 603-618 (1990)), and, for the introduction of exogenous DNA to a dicotyledonous plant cell, insertion of the nucleic acid of the present invention into the Ti plasmid of Agrobacterium and adding suitable ingredients to promote transformation thereby (Horsch et al., Science, 222, 496-498 (1984); DeBlock et al., EMBO J., 3, 1681-1689 (1984)). Other techniques are available for the introduction of exogenous DNA into a plant and/or a subset of its constituent cells, including electroporation, protoplast-mediated gene transfer, and silicon carbide crystal-mediated gene transfer. These various techniques are discussed in Genetic

Engineering News, vol. 14, no. 4 (Feb. 15, 1994) pages 1, 3, and 24, and are generally known in the art. Accordingly, plants that include polynucleotide sequences of the present invention may be generated using tissue culture, thereby growing transformed or transvected cells into plants, or conventional gene transfer directly into a plant. This line of plants having and expressing the nucleic acid of the present invention, accordingly, is established using conventional breeding techniques.

Detailed Description Paragraph Right (31):
Additionally, the inventive method further comprises (d) cross-pollinating a second plant by the first plant, wherein the second plant has a cytoplasmic male sterility factor that is compensated for by the action of the male fertility restorer gene product contained in the first plant. The cytoplasmic male sterility factor of the present invention is novel, although it may be related to or variants of any of the aforementioned C, S, or T cytoplasmic male sterility factors. The cytoplasmic male sterility factor of the present invention is selected as further described hereinbelow. Such a method may be used with any suitable species, preferably a species that reproduces by sexual means, such as maize, sorghum, and any of the various other plants aforementioned herein with respect to other embodiments of the present invention.

Detailed Description Paragraph Right (35):
The nucleic acid that is used in the inventive methods hybridize under at least moderately stringent hybridization conditions to a DNA that includes nucleotide sequences specific to a gene selected from the group of maize rf genes, as disclosed above. Such DNA preferably is flanked with a regulatory DNA sequence, such that a new plant variety, or cells thereof, derived from the aforementioned procedure, expresses its extra or altered rf genes in a developmentally and/or tissue-specific fashion.

Detailed Description Paragraph Right (36):
The method of the present invention is used to establish a new CMS plant variety of any suitable plant. Preferably, such a plant reproduces sexually, such as maize, soybean, rapeseed, canola, cotton, safflower, peanut, palm, beet, sorghum, and sunflower. More preferably, the method is used to produce new varieties of maize and sorghum; and most preferably, the method is used to produce new varieties of maize.

Detailed Description Paragraph Right (37):
The present invention also relates to any suitable organism comprising the vector comprising a nucleic acid comprising a nucleotide sequence that encodes a nuclear restorer gene product of a plant. A suitable organism can be any suitable plant, yeast, or bacteria, such as discussed hereinabove regarding suitable plants from which to isolate the restorer genes and regarding suitable hosts in which to insert the enriched or isolated nucleic acid of the present invention. Preferred organisms are sexually-reproducing plants, as described above. A seed-bearing plant that hosts a vector/nucleic acid construct can bear seeds that themselves include the construct, which seeds also constitute a preferred embodiment of the present invention.

Detailed Description Paragraph Right (40):
This example illustrates the use of two different transposon systems in maize for the selection of lines of plants that carry male fertility nuclear restorer genes.

Detailed Description Paragraph Right (41):
For tracking the mutagenesized chromosomes derived from crossing a transposon-carrying line to a genetically-marked non-transposon line, the following markers, arranged on the following restriction fragment length polymorphism (RFLP) maps of maize chromosomes 3 and 9, among others, were used. These maps are based on Wise et al., Theor. Appl. Genet., 88, 785-795 (1994), in which rf1 and rf2 were mapped to positions on chromosomes 3 and 9, respectively, with reference to closely-linked RFLP markers using five populations of maize. Analysis of RFLP markers was accomplished using conventional methods of DNA analysis (see Sambrook et al., supra). ##STR1##

Detailed Description Paragraph Right (43):
Based on the knowledge gained from the indicated classical genetic crossing studies, using the RFLP markers, stocks carrying certain transposon systems, i.e., Mutator, Cy, or Spm, were used to tag the genes of interest. Gene tagging using naturally-occurring transposons such as those just listed is described by Walbot, Ann. Rev. Plant Physiol.

Plant Mol. Biol., 43, 49-82 (1992). This method was used to identify previously mapped rf genes. Specifically, rf1 and rf2 were targeted in the following isolation plot cross using the method of Peterson, in Maize Breeding and Genetics (D. B. Walden, ed., John Wiley & Sons, New York, N.Y., 1978), pages 601-631 (the female parent is listed first in all crosses herein):

Detailed Description Paragraph Right (44):
The results of these crosses, including origin, size, and mutation rates in transposon populations screened for male-sterile mutations in maize, are presented below:

Detailed Description Paragraph Right (45):
Accordingly, transposon-generated mutants at the rf2 genetic locus were isolated and thus available for experiments directed to isolating the rf2 gene and/or cDNA.

Detailed Description Paragraph Right (46):
This example illustrates a method for isolating the rf2 gene from the rf2-m lines described in Example 1.

Detailed Description Paragraph Right (47):
A Mu1 transposon was shown to cosegregate with one of the alleles identified in Example 1 and Schnable et al., Genetics, 136, 1171-1185 (1994), i.e., rf2-m8122. As shown in FIG. 1A, cosegregation analysis was performed on a representative subset of 56 male-sterile (rf2-m8122/rf2-ref) and 49 male-fertile siblings (Rf2/rf2-m) from two segregating families derived from the cross: T cytoplasm Rf1/Rf1 rf2-R213/rf2-R213.times.Rf1/Rf1 Rf2/rf2-m8122. A 3.4 kilobase, Mu1-hybridizing EcoRI/HindIII restriction fragment is present in male-sterile siblings, but absent in male-fertile siblings in both of these segregating families. This DNA fragment was cloned into PF#9, which is an E. coli strain transformed by the plasmid pRf2 (see FIG. 2), as further discussed hereinbelow. Lanes are indicated as follows: OE, DNA isolated from the original male-sterile plant in which the rf2-m8122 allele was first identified. This plant had the genotype rf2-m8122/rf2-R213. GPP, GP, and P refer to the great-grandparent, grandparent and parent, respectively, of one of the segregating families. Each of these ancestors had the genotype rf2-m8122/Rf2. S and F refer, respectively, to male-sterile and male-fertile siblings within the segregating family.

Detailed Description Paragraph Right (48):
Using standard recombinant DNA techniques, total DNA from a single male-sterile plant that carried rf2-m8122 was subjected to preparative EcoRI/HindIII digestion and size-selected DNA was isolated for ligation into the lambda phage vector NM 1149 (Murray, The Bacteriophage Lambda, Hendrix ed. (1983)). The 3.4 kilobase EcoRI/HindIII fragment released from a Mu1-positive lambda phage (named .lambda.91 8122 #9) isolated from the resulting library was subcloned into plasmid vectors pBSK or pBKA (Stratagene), and named prf2. The prf2 plasmid was used to transform E. coli DH5.alpha. or XL1-Blue MRF', which transformed strain is referred to as PF#9. A restriction site map of the insert of the prf2 plasmid is shown in FIG. 2, wherein H stands for HindIII, D stands for DraI, Hc stands for HincII, P stands for PstI, Bg stands for BglII, and E stands for EcoRI. The location of the Mu1 insertion point is clearly marked therein between the second DraI and BglI restriction sites.

Detailed Description Paragraph Right (54):
Using .sup.32 P radiolabeled rf2 probes #1 and #2 (shown in FIG. 2) in combination, a cDNA library derived from mRNA isolated from immature maize tassels and cloned into .lambda.GT10 phage was screened, in accordance with methods well-known in the art (see Sambrook et al., supra; the cDNA library, named ts2, was a gift of S. Delaporta of Yale University). From a first screen, six putative positive plaques of phage were identified, which, upon a series of two rescreenings, were resolved to include three verified positive plaques. DNA from the positive phage were analyzed as to its ability to hybridize back to the aformentioned probes #1 and #2, and size of insert. The phage having the largest included insert was selected for subcloning that insert into plasmid pBSK and pBKA (Stratagene) for sequencing studies. The cDNA clone used for sequencing was named rf2 cDNA 6-2-8-1.

Detailed Description Paragraph Right (61):
Accordingly, for the identification of new CMS systems, one combines mutated

cytoplasms or existing variants of N that are "weakened" and mutated genes like rf2 that are associated with the mitochondria or cytosol. In combination, these two mutants serve as a CMS/restorer system. One can use such an approach in any species, and one could make as many CMS systems as desired. This invention has broad cross-species applicability in view of the close homology of SEQ ID NO:1 between proteins found in such evolutionarily distant organisms as maize and cows.

Detailed Description Paragraph Right (66):
The same strategy was used for the isolation of rf1 clones as was used for the isolation of rf2 clones, that being to use transposon lines to introduce tags that can be found with available transposon probes. As mentioned above, following a directed transposon tagging experiment, it is necessary to use linked markers (as identified in the map of Maize Chromosome 3 above) to distinguish between the newly induced mutants and the recessive allele used to uncover them. To identify an rf1 donor line with distinctive RFLP alleles flanking rf1, chromosome 3 RFLP fingerprints of five rf1 inbreds (W22, B37, Mo17, W64A, and B73) were obtained. These inbreds were RFLP finger printed by digesting their respective DNAs with 8 restriction endonucleases (BamHI, EcoRI, EcoRV, HindIII, KpnI, DraI, Bc1I, and BglII), followed by Southern blotting with RFLP markers that flank rf1. The results from this survey established that rf1-B37 can easily be distinguished from the Rf1-Ky21 or Rf1-WF9 alleles present in the Mutator population by using the restriction enzyme, DraI, in conjunction with umc10 and umc92, which flank rf1 (see above).

Detailed Description Paragraph Right (70):
To confirm the heritability of the putative male-sterile mutants and determine if the male-sterile phenotype is associated with the rf1 locus, the following experiments were performed on three of the putative male-sterile mutants: Exceptional male-sterile plants from Cross 4 (with the predicted genotype: T cytoplasm, rf1-Mu/rf1-B37, Rf2/Rf2) were crossed as shown in Cross 5: T rf1-Mu rg+/rf1-B37 rg+X T Rf1 Rg/rf1 rg+, wherein rg+refers to the normal wild type allele found in most maize lines.

Detailed Description Paragraph Right (73):
Co-segregation analysis with Mutator-specific hybridization probes may be performed for the purpose of identifying the individual Mu element inserted at an rf1-Mu allele: Because these rf1-Mu alleles have been isolated from Mutator populations (Cross 4), they may be associated with Mu element insertions. DNA flanking such Mu elements would have a high probability of representing rf1 sequences. Co-segregation analyses were used to identify the specific Mu elements responsible for a given mutation. The families resulting from Cross 6 and carrying rf1-Mu3207 and rf1-Mu3310 segregated 1:1 for male-sterile normal (rf1-Mu rg+/rf1-B37 rg+) and male-fertile ragged (Rf1Rg/rf1-B37 rg+) plants. DNA from each individual sibling was digested for such tests with HindIII and/or EcoRI). Mu1-specific sequences were used to probe genomic Southerns blotted with these DNAs. For each rf1-Mu allele, a Mu1-containing DNA fragment was identified that cosegregated with the mutant allele in over 40 progeny. These DNA fragments have been cloned using procedures as described in Example 2, and related procedures well-known in the art. DNA flanking a Mu1 element that co-segregated with male sterility in these crosses would have a high probability of representing rf1 sequences. Allelic cross-referencing experiments, as described above in Example 3, will be used to establish whether the cloned sequences indeed represent rf1.

Detailed Description Paragraph Table (1):

Maize populations used for mapping rf1 and rf2 with RFLP and visible markers.

Population Cross type	No. of progeny	Parent 1	Parent 2	Traits scored
Rf1 92 1267-68*	BC.sub.1 96	R213-T1g16 [Rf1rf1, rf2Rf2].sup..diamond-solid.	g16-N [rf1rf1,Rf2Rf2]	Rf1-mediated male fertility
G16 92 1140-43	F.sub.2 102.sup..tangle-solidup.	Q66-N [G16G16]	g16-N [g16g16]	g16
92 2117-118	RG1 92g 5029-63 TC 89 (6 selected).sup..circle-solid.	R213-T/Acc731	g16-N Rf1-mediated male fertility [Rf1rf1 rgRg rf2Rf2]	[rf1rf1 rgrg Rf2Rf2]
Rg1 RF2A 91g 6222-30	BC.sub.1 41	R213-T [Rf1Rf1,rf2rf2]	rf2-m 8904/R213-N	Rf2-mediated male fertility [rf1rf1,rf2Rf2]
RF2B 92 1101-05	BC.sub.1 903 (86 evaluated	R213-T/wx-m8	R213-N Rf1- and Rf2-mediated for RFLP markers) [rf1Rf1,Rf2rf2]	[Rf1Rf1,rf2rf2] male fertility

*Pedigree

numbers associated with this population .sup..diamond-solid. Parental genotype. See Wise et al, supra. .sup..tangle-solidup. Selected for homozygous g16. .sup..circle-solid. Ragged, malefertile plants carrying a recombination between the rg and rf1 loci were selected.

Detailed Description Paragraph Table (2):

rf2-m Mutation rate	alleles	Population	no./100,000	Population size	Subpopulation	Transposon donor source*	No. of isolated gametes
Mutator YA							
Mu.sup.4 outcross	(1220)	1	8,500	P	Mu.sup.2 outcross	(1120) 1	12,000 G
Mu.sup.1 outcrosses	(1212, 1215, 1218, 3219)		5,000	OB	Mu.sup.2 outcross	(1118) 0	12,000 B
Mu.sup.2 outcross	(1121, 4938)	0	9,700	m	Mu outcrosses	(1207, 1216, 1222, 1224) 0	3,100
Mutator population total		5	50,300	9.9	Cy	OA/BB 1230-1234, 3919-3921 1	28,000
Cy population total		1	28,000	3.6	Spm	CV Revertants from c1-m5 0	20,000
c1-m5 "Control"		1	80,000	Spm population total		1	100,000 1.0

*Transposon donor sources are indicated by the pedigree numbers, Mu outcross, Mu.sup.2 outcross, and Mu.sup.4 outcross are defined by Robertson, Mol. Gen. Genet., 191, 86-90 (1983).

Other Reference Publication (10):
Kamps, T., et al., Maize Genet. Coop. Newsl., 66, 45 (1992).

Other Reference Publication (29):
Rongnoparut et al. (1991) Isolation and characterization of a cytosolic aldehyde dehydrogenase encoding DNA from mouse liver. Gene vol. 101 pp. 261-265.

CLAIMS:

1. An enriched or substantially isolated nucleic acid comprising a nucleotide sequence that hybridizes at least under moderate stringency conditions to SEQ ID No:1 or SEQ ID No:3 and is capable or restoring male fertility to a plant having a cytoplasmic male-sterility trait.

3. The nucleic acid of claim 1, wherein said plant is selected from the group consisting of maize, soybean, rapeseed, canola, cotton, safflower, peanut, palm, sorghum, beet, and sunflower.

4. The nucleic acid of claim 3, wherein said plant is maize.

9. The method of claim 8, wherein said plant is maize or sorghum.